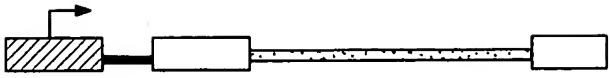
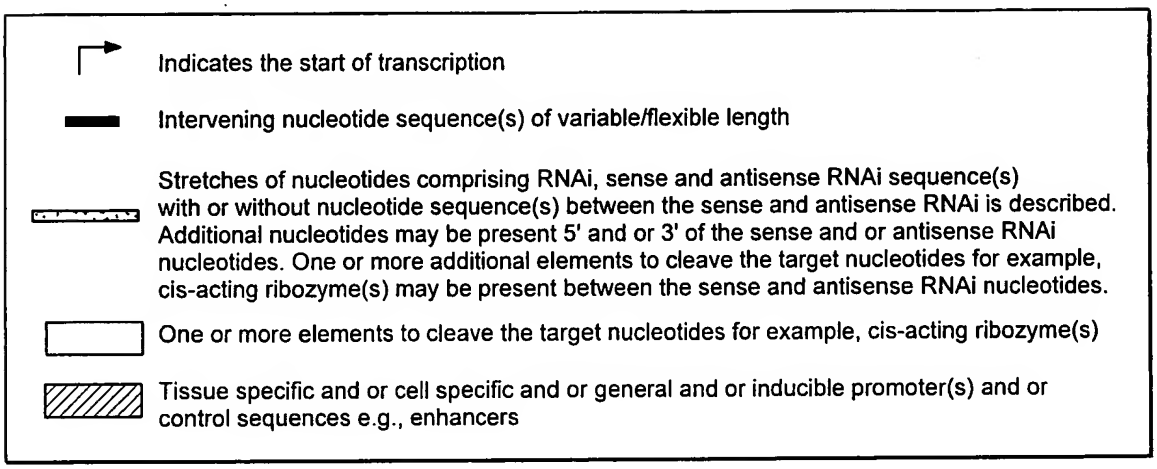
Design for RNAi expression

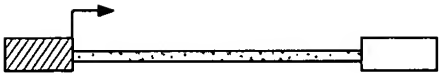




Expression of this cassette may be undertaken via inter alia a viral or non-viral vector, an artificial chromosome and or a stretch of DNA and or RNA

FIG. 1

Design for RNAi expression



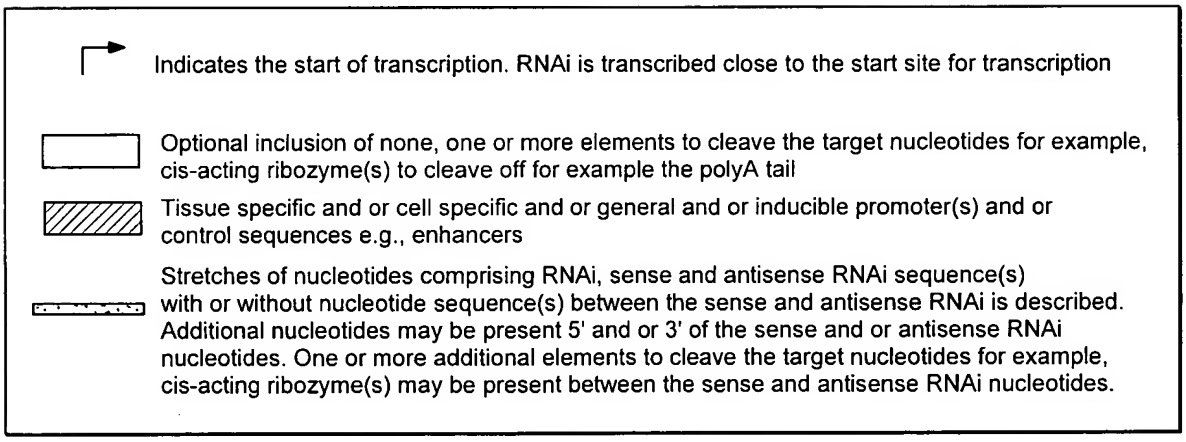


FIG. 2

Number	Gene	Reference	5' Sequences driving tissue specific expression
1	Rhodopsin, RHO gene	Gouras et al. (1994) Vis. Neurosci.11: 1227	Photoreceptor specificity can be achieved using various sequences 5' of the TSS of the rhodopsin promoter (from -222 to +70 and -2174 to +70). Expression in both rod and cone photoreceptor cells is observed using these 5' sequences
2	Alpha 1 type I collagen	Kalajuzic et al. (2002) Bone. 31: 654	Expression in osteoblasts can be achieved using, for example, 3.6 kb of the rat type collagen 1A1 promoter
3	Oligodendrocyte myelin glycoprotein	Solly et al. (1997) Neurochem. 68: 1705	657 bases of the 5' murine MOG can drive gene expression of a gene in an oligodendroglial cell line
4	Cone arrestin (CAR)	Zhu et al. (2002) Febs letters 524: 116	215 bases 5' fragment of the CAR gene can drive expression of a gene in retinoblastoma cells
5	Albumin	Postic et al. (1999) J. Biol. Chem. 274: 305	2.3 kilobases 5' of the albumin gene can drive expression of a gene in hepatocytes
6	Neuron specific enolase	Kowalski et al. (2001) Diabetes. 30: 425	1.8 kilobases of the neuron specific enolase drives gene expression in neurons
7	Human GnRH gene	Wolfe et al. Mol. Endo.16: 435	Expression in neurons can be achieved using 992 bases of the human GnRH gene promoter
8	alpha-actin promoter	Frauli et al. (2003) Med. Sci. Monit. 9: Br78	Human skeletal alpha-actin promoter (between -432 and +239) and beta-enolase enhancer (+504 and +637) can direct gene expression in skeletal muscle
9	Smooth muscle myosin heavy chain promoter	Franz et al. (1999) Cardiovasc. Res. 43: 1040	2.3 kilobases of the smooth muscle myosin chain promoter can direct expression to vascular tissue

Promoter sequence driving tissue specific gene expression

FIG. 3

CAACCACTACCTGAGCACCCAGTTCAAGAGACTGGGTGCTCAGGTAGTGGTTGTC

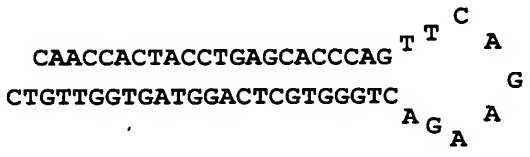


FIG. 4A

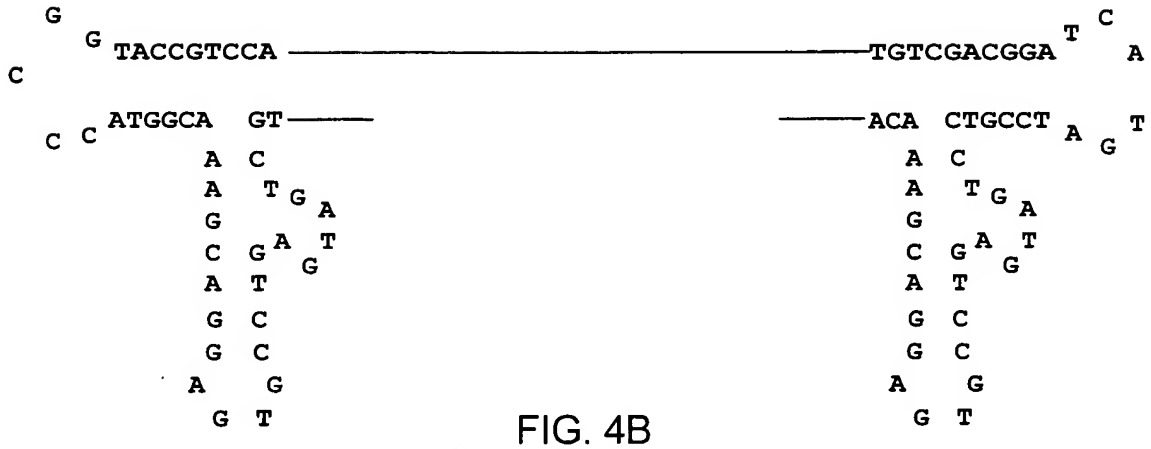

TACCGTCCA
TGTCGACGGA
ATGGCA GT
ACA CTGCCT

FIG. 4B

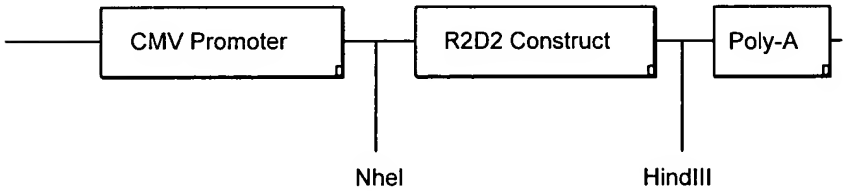

CMV Promoter
R2D2 Construct
Poly-A
NheI
HindIII

FIG. 4C

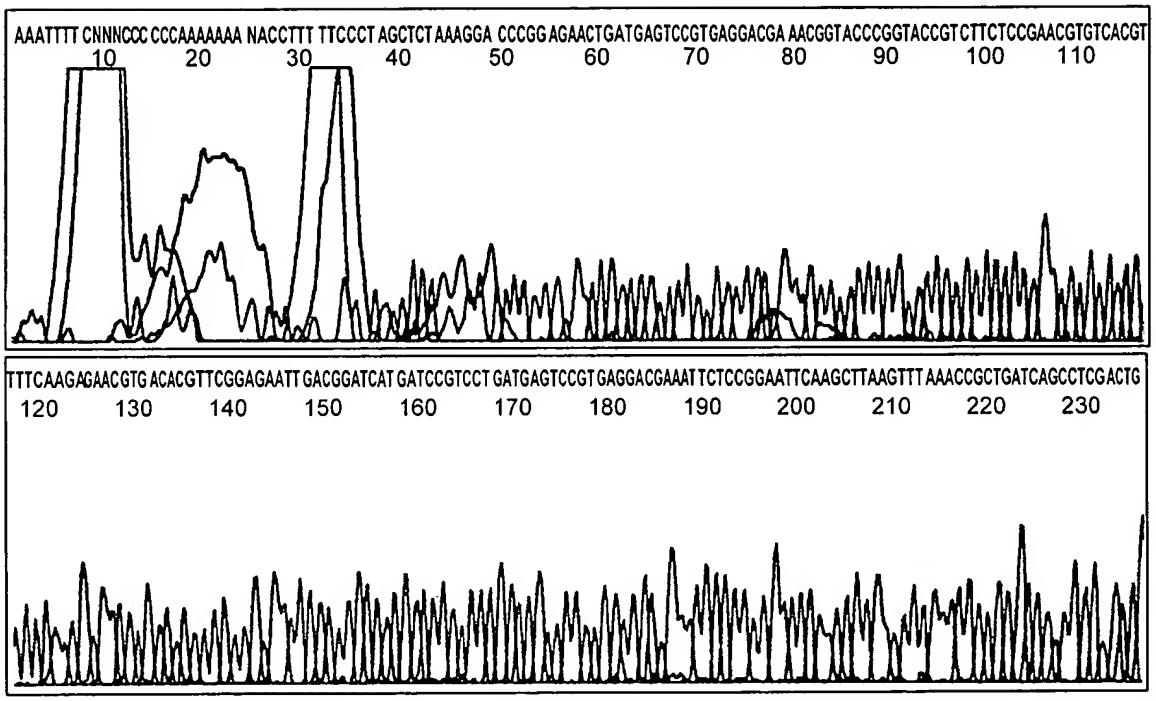


FIG. 4D-1

FIG. 4D-1
FIG. 4D-2

FIG. 4D

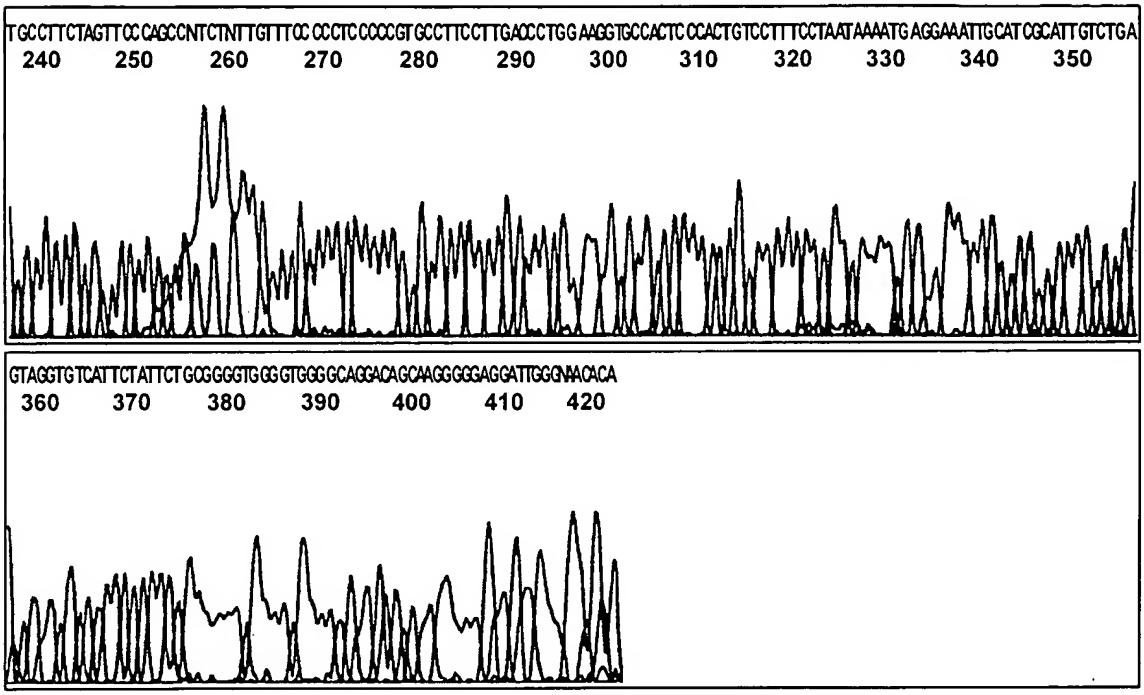

TGCCTTCTAGTTCCCAGCCNTCTNTTGTTTCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGA
240 250 260 270 280 290 300 310 320 330 340 350
GTAGGTGTCATTCTATTCTGCGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGNAACACA
360 370 380 390 400 410 420

FIG. 4D-2

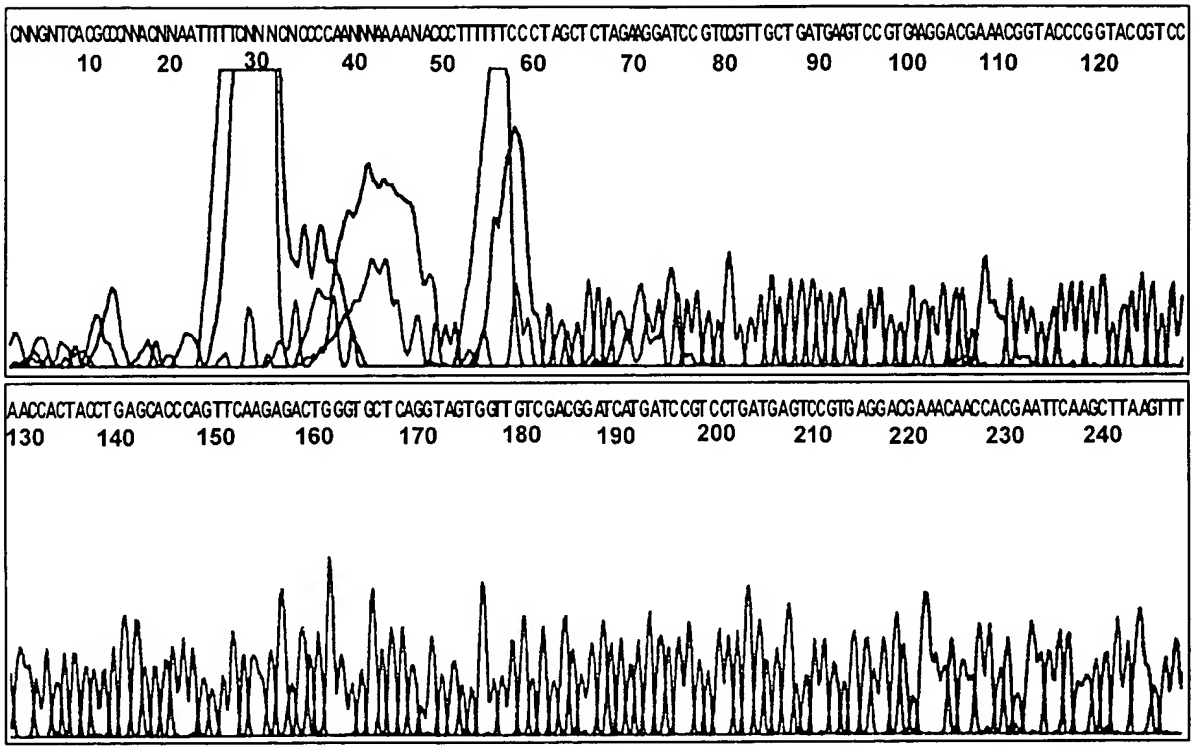


FIG. 4E-1

FIG. 4E-1

FIG. 4E-2

FIG. 4E

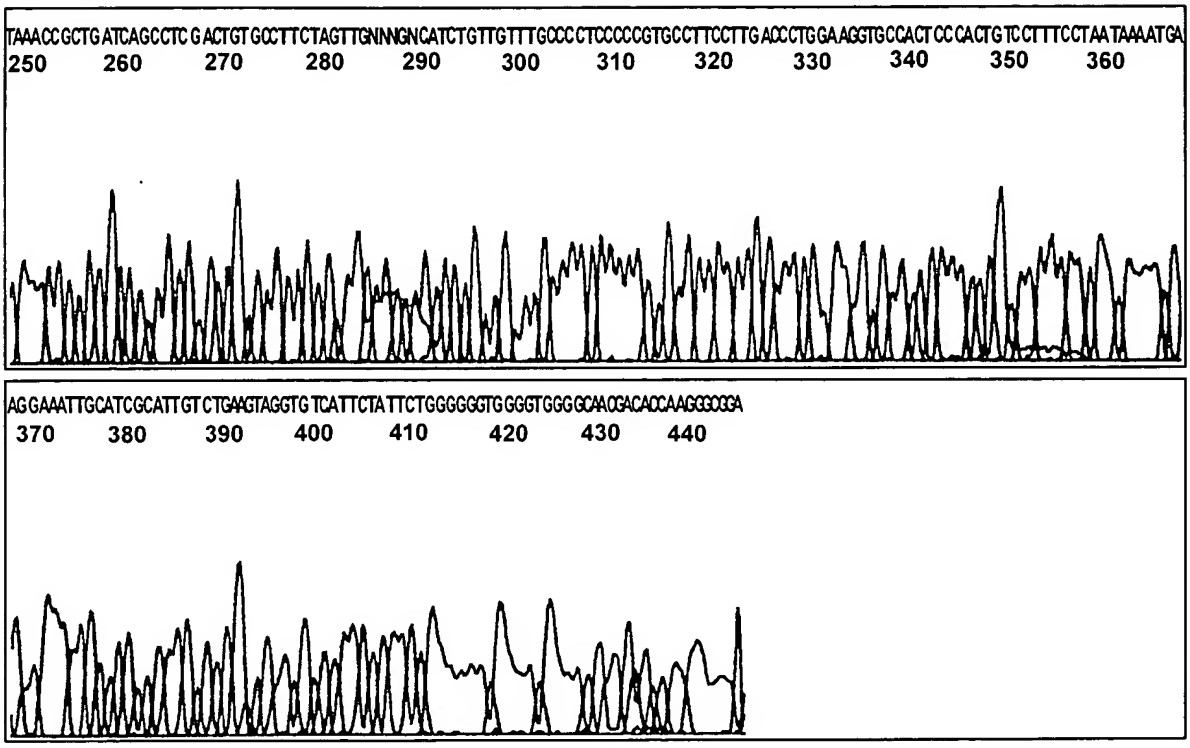


FIG. 4E-2

9/15

FIG. 4F-1

FIG. 4F-2

FIG. 4F

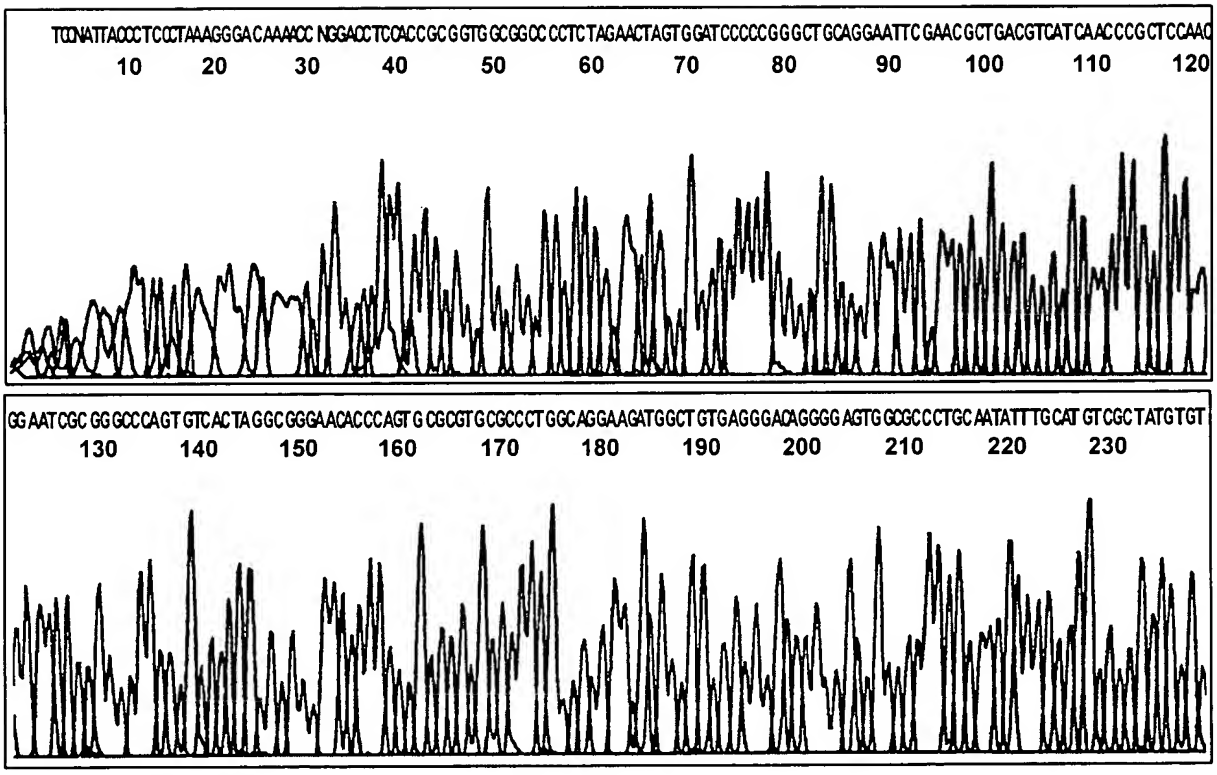

TCCNATTACCCTCCCTAAAGGGACAAACCNGGACCTCCACCGCGGTGGCGGCCCCTCTAGAACTAGTGGATCCCCCGGGCTGCAGGAATTCGAACGCTGACGTCATCAACCCGCTCCAAC
10 20 30 40 50 60 70 80 90 100 110 120
GGAATCGCGGGCCCAGTGTCACTAGGCGGGAACACCCAGTGCGCGTGCGCCCTGGCAGGAAGATGGCTGTGAGGGACAGGGGAGTGGCGCCCTGCAATATTTGCATGTCGCTATGTGT
130 140 150 160 170 180 190 200 210 220 230

FIG. 4F-1

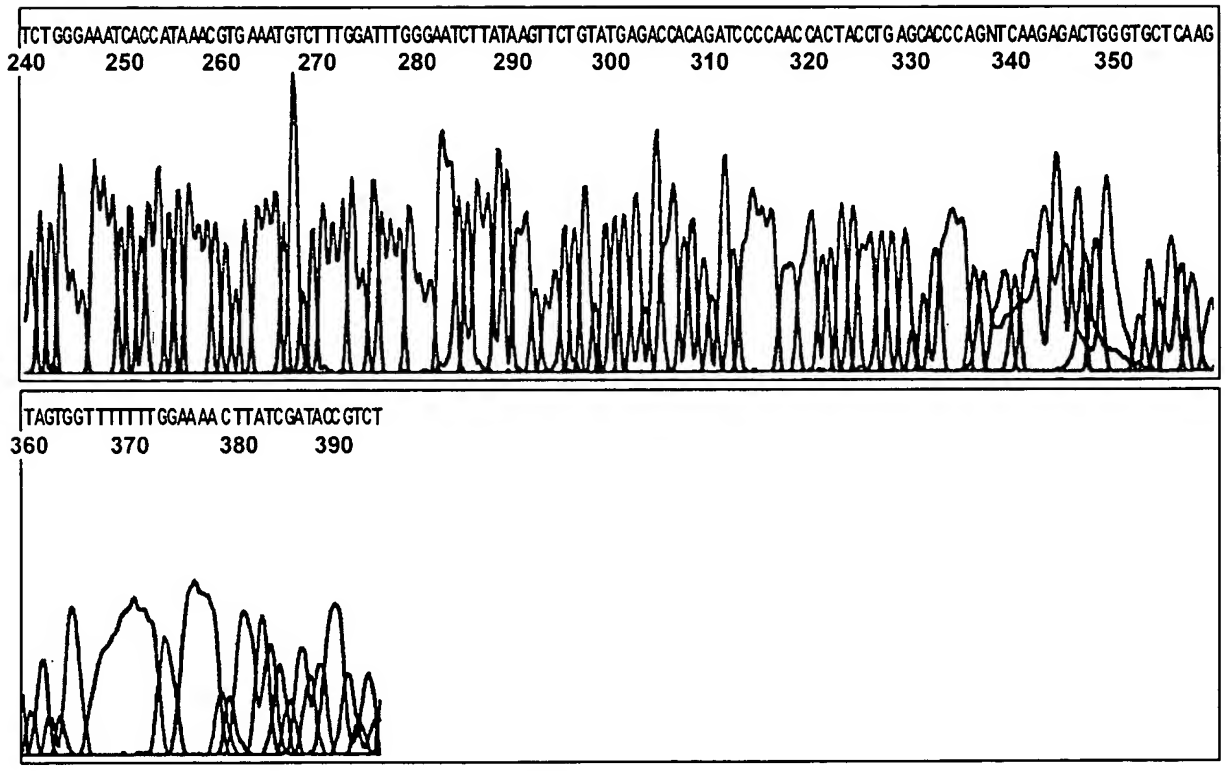

TCTGGGAAATCACCATAAACGTGAAATGTCTTTGGATTTGGGAATCTTATAAGTTCTGTATGAGACCACAGATCCCCAACCACTACCTGAGCACCCAGNTCAAGAGACTGGGTGCTCAAG
240
250
260
270
280
290
300
310
320
330
340
350
TAGTGGTTTTTTTGGAAAACTTATCGATACCGTCT
360
370
380
390

FIG. 4F-2

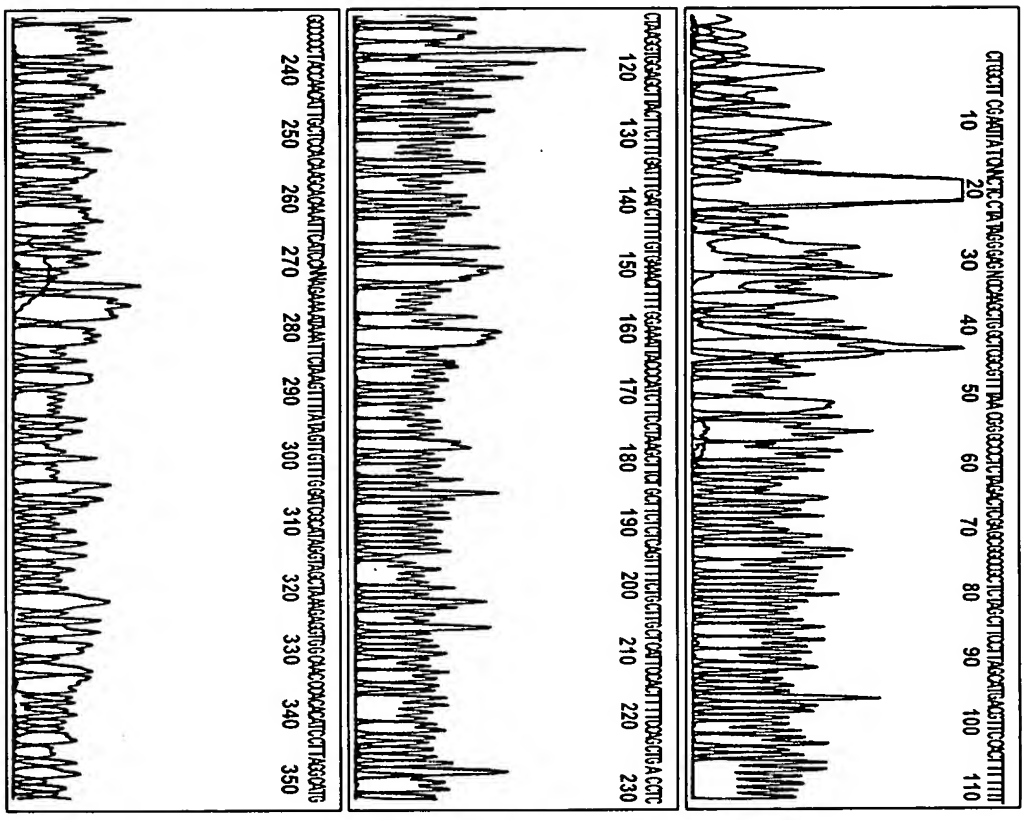


FIG. 4G

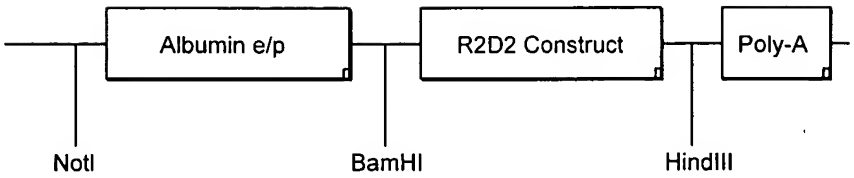


FIG. 5A

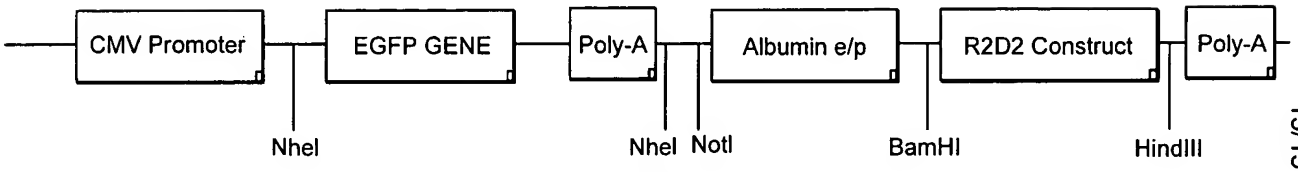


FIG. 5B

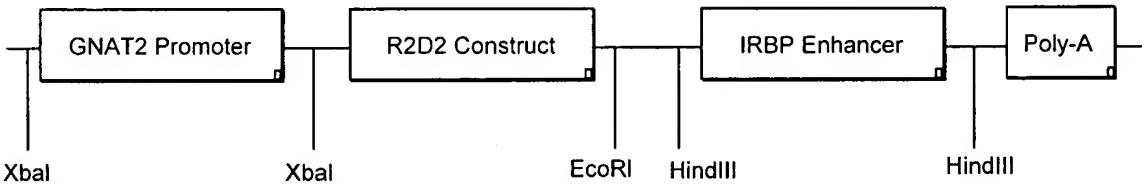


FIG. 5C

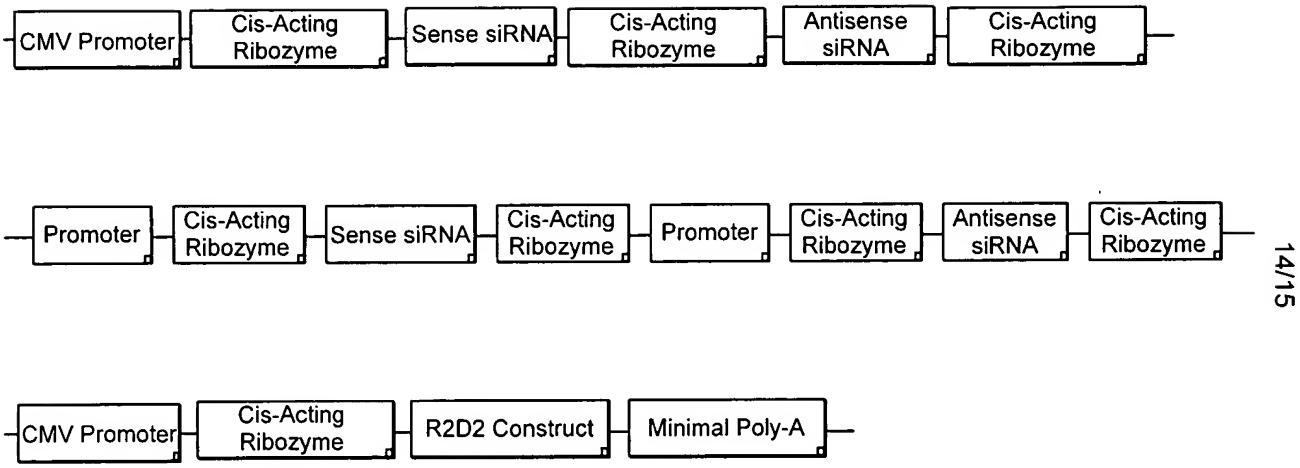

CMV Promoter
Cis-Acting Ribozyme
Sense siRNA
Cis-Acting Ribozyme
Antisense siRNA
Cis-Acting Ribozyme
Promoter
Cis-Acting Ribozyme
Sense siRNA
Cis-Acting Ribozyme
Promoter
Cis-Acting Ribozyme
Antisense siRNA
Cis-Acting Ribozyme
CMV Promoter
Cis-Acting Ribozyme
R2D2 Construct
Minimal Poly-A

FIG. 6

FIG. 7A

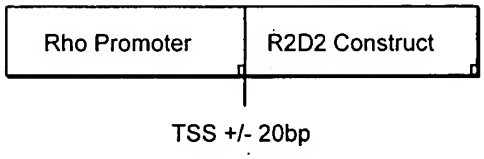


FIG. 7B

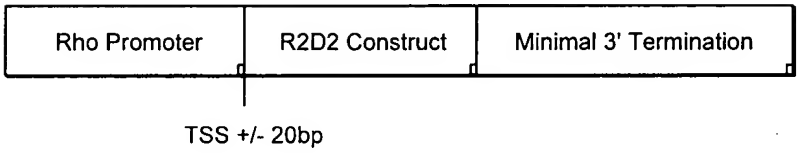


FIG. 7C